

# Comparative Field Stability of Selected Entomopathogenic Virus Formulations

M. R. MCGUIRE,<sup>1</sup> P. TAMEZ-GUERRA,<sup>2</sup> R. W. BEHLE,<sup>3</sup> AND D. A. STREETT<sup>4</sup>

Bioactive Agents Research Unit, USDA-REE-ARS, National Center for Agricultural Utilization Research,  
1815 N. University Street, Peoria, IL 61604-3902

J. Econ. Entomol. 94(5): 1037-1044 (2001)

**ABSTRACT** Nucleopolyhedroviruses originally isolated from *Anagrapha falcifera* (Kirby) and *Autographa californica* (Speyer) were formulated with various ingredients using a spray dry method and tested for residual field activity in Illinois and Mississippi. In Mississippi, field tests were conducted on cotton in 1997, whereas in Illinois tests were conducted on cabbage in 1997 and 1998. Within 24 h, significant differences were observed among formulations in all tests. Unformulated virus had significantly less insecticidal activity than formulated virus and formulations containing lignin retained activity significantly longer than other formulations. Relatively small amounts of Blankophor BBH, when encapsulated within the formulation, did not greatly enhance ( $>10\times$ ) insecticidal activity based on  $LC_{50}$  determinations nor prolong insecticidal activity based on field evaluations. In most tests,  $>50\%$  activity remained in formulations containing lignin, whereas unformulated virus retained  $<50\%$  activity within 24 h after application.

**KEY WORDS** Lepidoptera, AcMNPV, AfMNPV, formulation, solar stability, field activity

LACK OF FIELD stability of entomopathogenic viruses after application has long been considered a primary factor limiting commercialization of these important insect microbial control agents (Bull et al. 1976, Jaques 1977). Despite extensive research efforts by private and public sector scientists, practical and economic formulation techniques and materials have not been found to significantly lengthen the effective residual field activity of viruses. Granular formulations of entomopoxvirus (McGuire et al. 1991) and *Heliothis* NPV (Ignoffo et al. 1991) based on cornstarch and solar protectants, applied dry, had greater residual activity than unformulated virus, but granular and dust formulations are not readily accepted by the end user. Bull et al. (1976) and Ignoffo et al. (1997) demonstrated that carbon could significantly extend the activity of virus in response to simulated sunlight. Spray tank additives such as optical brighteners have been shown to enhance the activity of several nucleopolyhedrovirus by synergizing toxicity and/or providing protection from sunlight degradation. Residual activity can effectively be extended by either of these mechanisms, making addition of a brightener a logical choice as a spray additive when used for control of

lepidopterous pests (Shapiro 1992, Shapiro and Robertson 1992, Webb et al. 1996, Li and Otvos 1999). However, industry has been reluctant to adopt protective adjuvants whose effectiveness is related to the volume of spray applied. For example, an adjuvant that is effective at 2% solids would require 10 times less material when added to 10 liters of water versus 100 liters of water. Companies would prefer to supply formulations that can be applied at specific rates per unit area and which are independent of spray volume.

A new spray-dried formulation to microencapsulate *Bacillus thuringiensis* with protective ingredients (Tamez-Guerra et al. 1996) provided increased resistance to solar degradation of *B. thuringiensis*. Subsequent field tests demonstrated control of *Epilachna varivestis* (Mulsant) in beans (Tamez-Guerra et al. 1999). The technique of spray drying virus with protective ingredients was recently developed and has undergone extensive laboratory testing (Tamez-Guerra et al. 2000a). The particles that are formed maintain their shape in the spray tank and will pass through standard hydraulic spray nozzles. By binding protective ingredients to the active ingredient, the need for a spray volume-dependent adjuvant is eliminated. In this manuscript we report the results from 2 yr of field tests of formulations made with different protective ingredients, different viruses and different field and bioassay conditions. We surmise that a range of formulation and testing conditions should facilitate selection of an efficacious and long-lasting virus formulation.

This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or recommendation by the USDA for its use.

<sup>1</sup> Current Address: Western Integrated Cropping Systems Research Unit, 17053 Shafter Avenue, Shafter, CA 93263.

<sup>2</sup> Dep. De Microbiología e Inmunología, fac. De Ciencias Biológicas, UANL, San Nicolas de los Garza, Nuevo Leon, Mexico.

<sup>3</sup> To whom reprint requests should be addressed.

<sup>4</sup> Southern Insect Management Laboratory, USDA-ARS, P.O. Box 346, Stoneville, MS 38776.

**Table 1.** Ingredients used to prepare spray-dried virus formulations for testing field residual activity; amounts are based on a 1-liter dryer feed stock mix

Year	Formulation	Lignin, g	PCF, <sup>a</sup> g	NCF, <sup>b</sup> g	CaCl <sub>2</sub> , g	Blankophor BBH, g	TiO <sub>2</sub> , g	Tween, ml	Sugar, g	2-propanol, ml	PIB <sup>c</sup> /g (×10 <sup>9</sup> )
1997	Lignin <sup>d</sup> -flour	65	35		13			25		100	0.9 1.2
	Lignin-composite	37	25	25	8			75	37	150	0.7 1.2
	BBH-flour		60		10	1		25			0.6 1.1
	BBH-composite		25	25	10	1.1		25	50		0.3 1.2
	Lignin only	25			5			25		80	0.8 0.8
	BBH only					5		0.5			2.4 0.8
1998	Lignin <sup>e</sup> -flour	60	30		6						2.2
	Lignin-flour-TiO <sub>2</sub>	46	23		5		23				2.2
	Lignin only	85			9						2.2
	Lignin-TiO <sub>2</sub>	60			6		30				2.2

<sup>a</sup> Pregelatinized corn flour, Flour 961; Illinois Cereal Mills, Paris, IL.

<sup>b</sup> Nixtamalized corn flour, Maseca; Guadalupe, N. L. Mexico.

<sup>c</sup> Polyhedral inclusion bodies per gram of dry ingredients. The first number is for formulation made with AcMNPV, the second number with A/MNPV.

<sup>d</sup> Potassium lignate; made by mixing 90.0 g of kraft lignin (Westvaco, Charleston Heights, SC) in 200 ml of deionized water and 11.0 g of potassium hydroxide. The mixture was dried under the hood and ground to pass through 30-mesh sieve.

<sup>e</sup> Sodium lignate PC1307, Westvaco.

## Materials and Methods

**Virus Sources.** Multiple embedded nucleopolyhedrovirus polyhedral inclusion bodies (PIB) originally isolated from *Autographa californica* (Speyer) (AcMNPV) were produced in vivo and supplied by DuPont (Wilmington, DE) for formulation without further propagation. A virus originally isolated from *Anagrapha falcifera* (Kirby) (A/MNPV) was obtained from biosys (now Thermo Trilogy, Columbia, MD) and then propagated in our laboratory in third-instar *Trichoplusia ni* (Hübner) (Tamez-Guerra et al. 2000a) for 1997 experiments. For experiments in 1998, four separate production lots of A/MNPV were provided by Thermo Trilogy and each of four formulations were made with each lot for a total of 16 formulations (see below).

**Formulation Preparation.** In 1997, six formulations were made with each virus. Materials used to make these experimental formulations included water-soluble lignin (see below), Flour 961 (Illinois, Cereal Mills, Paris, IL), nixtamalized corn flour (NCF, Maseca, Guadalupe, N.L., Mexico), Blankophor BBH, (Bayer, Kansas City, MO), TiONIA RCL9 titanium dioxide (Millenium Inorganic Chemicals, Hunt Valley, MD) calcium chloride (Fisher, Fairlawn, NJ), sucrose, Tween 85 (T. J. Baker Chemical, Phillipsburg, NJ), and 2-propanol (Fisher). Formulations were made with a soluble Kraft lignin (Westvaco, Charleston Heights, SC) made previously by mixing 90 g lignin in 200 ml deionized water and 11 g potassium hydroxide. This lignin solution was air dried under a fume hood and ground to pass a 30-mesh sieve to provide a water soluble lignin powder used to make the 1997 formulations. Descriptive names and ingredients for each formulation are listed in Table 1 with the specific ingredients used for production. Final concentrations of dry ingredients were between five and 10% wt:vol in the final dryer feed stocks. General mixing procedures began with dissolving the lignin in deionized water (2,000 ml) using a blender for agitation. Once

dissolved, flour(s), Blankophor, titanium dioxide, sugar, Tween, and virus (75 g AcMNPV or A/MNPV) were added and mixed with the blender. After these ingredients were mixed, calcium chloride (in a 10% wt:vol solution) was added slowly with continuous agitation to avoid gelling the mixture. When these mixtures were too thick to pump into the spray dryer at this stage, propanol was added to improve flow. The spray drying conditions were reported by Tamez-Guerra et al. (2000a). A pilot plant scale atomizer (Niro, Columbia, MD) was used to dry all formulations. Samples were fed into the spray dryer at 8 ml/min in 1997 (20 ml/min in 1998). Inlet temperature was 100°C in 1997 or 130°C for the higher feed rate in 1998. Outlet temperature was 65–70°C, and air pressure was 4.5 kg/cm<sup>2</sup>. Product residence time in the dryer was a matter of seconds, generally less than 10 s. Formulations were stored at 4°C until use.

In 1998, the lignin used to make the formulations was PC-1307 produced by Westvaco. Four formulations were made with only A/MNPV using simplified formulations (Table 1). The order of mixing of the formulation ingredients was the same as described above. Drying conditions were altered to speed the process by increasing the flow rate to 20 ml/min and inlet temperature to 130°C while maintaining the outlet temperature at 65–70°C.

All formulations were assayed against laboratory reared *T. ni* neonates to determine LC<sub>50</sub>. In 1997, formulations were assayed using a cotton leaf disk technique reported by McGuire et al. (1997). Briefly, a suspension of formulation was spread onto a pre-marked area of cotton leaf and allowed to dry. Ten larvae were then confined on each of five excised disks per treatment and allowed to feed 24 h after which six live larvae from each leaf disk were transferred to individual diet cups (37 ml) containing about 3 ml artificial diet. Larvae were stored in a dark incubator at 28°C and 50% RH. Mortality was assessed 7 d after initial exposure to leaf disks. In 1998, a droplet assay

modified from Hughes and Wood (1981) was used to determine  $LC_{50}$ . Larvae were allowed to feed on droplets of colored sugar solution (0.1% wt:vol FD&C Blue 1, Uklton Davis, Cincinnati, OH; 2.0% sucrose) containing concentrations of virus formulations. The colored feeding solution contained 0.3% wt:vol  $NaCO_3$  to dissolve the microparticles. We found that this procedure was necessary to alleviate settling by the formulation in the droplet (Behle et al. 2000). Approximately 10 min after larvae were placed in the dish with the droplets, 30 fed larvae (as evidenced by blue color in the gut) from each treatment were transferred to individual diet cups containing artificial diet. Mortality was assessed 7 d later. To analyze data, a Basic program based on Finney (1971) was used to calculate  $LC_{50}$  based on five concentrations of virus and 30 larvae per concentration dose. No attempt was made to standardize virus applications in the field tests based on the  $LC_{50}$  results.

Formulations tested in Mississippi (except the "Bell" formulation) were prepared at the National Center for Agricultural Utilization Research and stored refrigerated before being sent on ice via overnight mail. The "Bell" formulation was made in Mississippi with a sample of the same batch of virus used to prepare the other formulations in the experiment. The Bell formulation was not spray dried and consisted of 1.5% crude cottonseed oil (Yazoo Valley Oil Mill, Yazoo City, MS), 0.04% Tween 80 (Baker Chemical Company, Phillipsburg, NJ), and 3% sucrose (Baker). This mixture was added to the spray tank containing virus. The glycerin formulation used in 1998 was made by diluting unformulated and non-spray-dried virus from each production lot to  $2 \times 10^9$  PIB/ml with water and then adding an equal volume of glycerin (Fisher, St. Louis, MO) to make a final formulation with  $1 \times 10^9$  PIB/ml in 50% glycerin.

**Field Applications and Bioassay Procedures.** *Mississippi.* Each plot consisted of six rows of cotton (DP 5409) 15 m long. AcMNPV formulations were applied to each of four replicated plots in a completely randomized design. Cotton had been planted in May and was a few days from cutout. Treatments were applied 11 August 1997 to the middle four rows at a rate of  $4.9 \times 10^{12}$  PIB/ha ( $2 \times 10^{12}$  PIB/acre) in a spray volume of 56 liters/ha (6 gal/acre) via a tractor-mounted sprayer. Because formulations had different PIB concentrations, the amount of each formulation added to the spray tank was adjusted to apply equal PIB concentrations per plot. One 12× TeeJet nozzle (Spraying Systems, Wheaton, IL) was directed over the top of each row and two nozzles were suspended between the rows to maximize coverage. Five leaf samples were removed from the top third of plants in each of the middle two rows immediately after spray deposits had dried and 24, 48, and 72 h after application. A sterilized cork borer was used to remove three 1.5-cm-diameter disks from each leaf. Disks (30 per plot) were transferred to individual diet cups containing a moistened piece of filter paper. A single 5- to 6-d-old *Heliothis virescens* (F.) larva was added to each cup. After 48 h, larvae were transferred to artificial diet

and held for an additional 8 d before mortality was assessed.

*Illinois.* Tests were done twice in 1997 (once each with formulations of AcMNPV and A/MNPV) and twice in 1998 (both times with A/MNPV). For both years, procedures were the same and an unformulated virus was used as a control. Cabbage (cultivar 'Bravo') was transplanted approximately 1 mo before the first field test. Each plot was one row consisting of 8–10 plants spaced  $\approx 30$  cm apart. Rows were spaced one m apart. Applications were made with a  $CO_2$ -charged backpack sprayer rigged with three nozzles, one directed over the top and one on each side of the row. The sprayer was calibrated to deliver  $\approx 234$  liters of water per ha (25 gal/acre). In 1997, formulations were mixed with water to provide  $1.2 \times 10^{12}$  PIB/ha ( $5 \times 10^{11}$  PIB/acre). Each formulation was mixed once and applied to each of four plots in a randomized complete block design. In 1998, each formulation was made with each of four lots of virus and applied at the rate of  $2.5 \times 10^{12}$  PIB/ha ( $1 \times 10^{12}$  PIB/acre). Formulations for each lot were applied to plots in respective blocks in a randomized complete block design. Thus, each lot of virus represented a replication. In 1997, cabbage leaves were collected 2, 26, 50, and 74 h after application. In 1998, leaves were collected 2, 6, 26, 50, and 74 h after application for the first experiment but after 2, 26, 50, 74, and 96 h in the second experiment. For each collection, five leaf disks were removed from the five middle plants in each plot and placed individually into 5 cm diameter plastic petri dishes containing a piece of filter paper. Ten *T. ni* neonates were added to each dish which was then capped with a sealing lid. After 48 h, six live larvae from each dish were transferred to artificial diet and held an additional 5 d before mortality was assessed.

**Statistical Analysis.** For each day of each field experiment, analysis of variance (ANOVA) was done on untransformed percentage mortality and differences among treatment means were determined using a protected least significant difference (LSD) test (Analytical Software 1997). Untreated control mortalities were generally  $<10\%$  for each sampling period, except where noted, and were not included in the analyses.

## Results

**Viability of Spray-Dried Formulations.** All spray-dried formulations were tested for activity in the laboratory after processing. In 1997, the two lignin-based formulations of AcMNPV had higher ( $\approx 3\times$ )  $LC_{50}$  than the other formulations, suggesting some loss of activity due to the materials used in the spray drying techniques (Table 2). These lignin formulations had significantly higher  $LC_{50}$ s than the BBH-flour formulation based on nonoverlap of 95% confidence intervals. This loss of activity was not observed, however, when the same ingredients were used to make A/MNPV formulations. The lignin-only and Blankophor BBH-only formulations were prepared immediately before the field test and were not included in these  $LC_{50}$  determinations.

Table 2. Initial insecticidal activity based on a treated cotton leaf-disk assay of spray-dried formulations of *AcMNPV* and *A/MNPV* prepared in 1997

Virus	Formulation	Slope (SE)	LC <sub>50</sub> (PIB × 10 <sup>6</sup> /ml)	95% CI (PIB × 10 <sup>6</sup> /ml)
<i>AcMNPV</i>	Lignin-flour	1.01 (0.19)	5.26	2.80–14.8
	Lignin-Composite	0.84 (0.17)	5.91	2.79–22.8
	BBH-flour	1.28 (0.17)	1.28	0.80–2.19
	BBH-composite	1.36 (0.21)	1.97	1.27–3.33
	Unformulated	1.07 (0.16)	1.55	0.93–2.86
<i>A/MNPV</i>	Lignin-flour	1.29 (0.19)	1.81	1.15–3.13
	Lignin-Composite	1.14 (0.17)	1.13	0.69–1.96
	BBH-flour	1.13 (0.18)	2.29	1.37–4.56
	BBH-composite	0.90 (0.15)	1.43	0.80–2.97
	Unformulated	1.08 (0.18)	1.93	1.14–3.80

See Table 1 for formulation composition.

In 1998, no significant differences occurred within lots 1 and 3 for initial insecticidal activity. However, within lot 2, the glycerin formulation had significantly higher activity ( $\approx 2.4\times$ ) than the lignin-flour formulation (Table 3). But the LC<sub>50</sub> determination for each of these two formulations had  $\chi^2 > 8$  (df = 3), suggesting a lack of goodness-of-fit to the probit model. In lot 4, the lignin-flour-TiO<sub>2</sub> formulation had significantly less activity than the lignin-flour formulation. The LC<sub>50</sub> data for 1998 were analyzed by ANOVA. Mean LC<sub>50</sub>s for virus lots (reps) were significantly different ( $F = 9.5$ ; df = 3, 15;  $P = 0.0089$ ), but mean LC<sub>50</sub>s for formulations were not significantly different ( $F = 0.75$ ; df = 5, 15;  $P = 0.6006$ ). LSD to separate the means for the lots indicated that lot 2 had a significantly higher LC<sub>50</sub> than the other three virus lots, which were not significantly different. All formula-

tions were applied in the field at a virus concentration based on the calculated PIB per gram of formulation solids. The overall differences between LC<sub>50</sub>s in 1997 and 1998 are attributed to the difference in bioassay technique.

**Field Activity.** Due to significant experiment  $\times$  treatment and treatment  $\times$  day interactions, results from each day of each experiment were analyzed separately.

**Mississippi.** Significant differences occurred among the formulations 24 h after application to cotton (Table 4). The two lignin-based formulations caused higher mortality of *H. virescens* than the two Blankophor-BBH and the “Bell” formulations. These differences were also observed for leaf samples collected 48 and 72 h after application. Rainfall was recorded between the 24- and 48-h sample collections (50 mm) and

Table 3. Initial insecticidal activity based on a droplet feeding assay of spray-dried formulations of *A/MNPV* prepared in 1998

Virus lot	Formulation	Slope (SE)	LC <sub>50</sub> (PIB × 10 <sup>4</sup> /ml)	95% CI (PIB × 10 <sup>4</sup> /ml)
1	Lignin only	1.68 (0.26)	10.67	6.95–15.20
	Lignin-flour	1.53 (0.25)	8.55	5.09–12.64
	Lignin-TiO <sub>2</sub>	1.71 (0.29)	7.05	4.25–10.22
	Lignin-flour-TiO <sub>2</sub>	1.39 (0.23)	8.67	4.86–13.24
	Glycerin	1.60 (0.28)	7.31	4.21–10.96
	Unformulated	1.28 (0.23)	6.74	3.25–10.85
2	Lignin only	1.99 (0.30)	15.98	10.54–20.71
	Lignin-flour	1.72 (0.24)	17.05 <sup>a</sup>	11.69–23.96
	Lignin-TiO <sub>2</sub>	1.85 (0.28)	11.70	7.93–16.44
	Lignin-flour-TiO <sub>2</sub>	2.21 (0.34)	14.84	10.64–20.26
	Glycerin	1.24 (0.22)	7.08 <sup>a</sup>	3.47–11.41
	Unformulated	1.58 (0.23)	14.24	9.32–20.58
3	Lignin only	0.95 (0.20)	4.37	1.21–8.57
	Lignin-flour	0.98 (0.20)	5.65	1.91–10.47
	Lignin-TiO <sub>2</sub>	1.20 (0.21)	7.24	3.45–11.80
	Lignin-flour-TiO <sub>2</sub>	0.63 (0.19)	1.65	0.03–5.46
	Glycerin	1.25 (0.24)	4.41	1.74–7.53
	Unformulated	0.83 (0.18)	6.20	1.78–12.19
4	Lignin only	0.67 (0.18)	4.93	0.58–11.89
	Lignin-flour	0.95 (0.22)	2.71	0.48–5.92
	Lignin-TiO <sub>2</sub>	0.88 (0.18)	9.64 <sup>a</sup>	3.78–17.49
	Lignin-flour-TiO <sub>2</sub>	1.27 (0.21)	12.36	7.14–19.10
	Glycerin	1.14 (0.23)	4.61	1.66–8.20
	Unformulated	0.80 (0.18)	4.06	0.76–8.94

See Table 1 for formulation composition.

<sup>a</sup>  $\chi^2 > 8$ ; (df = 3);  $P < 0.05$ .

Table 4. Residual insecticidal activity based on percentage mortality of *H. zea* when placed on cotton leaves harvested after application of AcMNPV formulations at  $4.9 \times 10^{12}$  PIB/ha in 56 liters/ha to field grown cotton in Mississippi, 11 August 1997

Formulation	Hours after application			
	0	24	48	72
Lignin-flour	94.4	92.2a	52.2a	16.7a
Lignin-Composite	94.6	87.6a	40.1a	14.5ab
BBH-flour	94.3	62.2b	10.4b	4.7c
BBH-composite	96.9	54.0b	4.0b	6.3bc
Bell	93.4	57.8b	10.0b	2.8c
F (df = 4, 15)	0.09	7.15	16.01	3.77
CVC	NA	20.0	16.2	9.6
SEC	NA	9.4	7.6	4.5

Means in a column followed by the same letter are not significantly different, LSD,  $P = 0.05$ . (Means for samples collected at 0 h were not significantly different.) Untreated control mortalities were less than 10% for each sample period. See Table 1 for formulation composition; see text for composition of "Bell" formulation. NA, not applicable; CVC, critical value for comparison ( $P = 0.05$ ). SEC, standard error of comparison.

between the 48- and 72-h samples (80 mm). By 72 h after application, all treatments had lost insecticidal activity.

*Illinois.* No significant rainfall occurred during either test conducted in Illinois in 1997.

*AcMNPV.* Immediately after application, significant differences were observed among the formulations applied to cabbage (Table 5). The Blankophor BBH-composite and Blankophor BBH-only formulations caused significantly higher *T. ni* mortality than the lignin-flour and lignin-only formulations. These early differences may be related to the differences in LC<sub>50</sub> observed before the field test. Within 26 h, these differences were not detectable, suggesting that the lignin-based formulations protected virus better than the Blankophor BBH formulations. Unformulated virus lost activity within the first day of exposure to field

Table 5. Residual activity based on percentage mortality of *T. ni* when placed on cabbage leaves harvested after application of AcMNPV formulations at  $1.2 \times 10^{12}$  PIB/ha in 234 liters/ha to field-grown cabbage plants, Peoria, IL, 23 June 1997

Formulation	Hours after application			
	2	26	50	74
Lignin-flour	59.6cd	35.8ab	8.6bc	0
Lignin-composite	65.0bcd	43.7ab	11.1bc	0.8
Lignin only	53.0d	41.5ab	10.0bc	3.4
BBH-flour	71.7bc	31.7bc	22.0ab	5.2
BBH-composite	88.3a	52.5a	27.5a	5.9
BBH only	76.4ab	53.3a	9.7bc	5.3
Unformulated	60.6cd	17.0c	2.0c	2.5
F (df = 6, 18)	5.4	4.6	3.1	0.9
CVC	15.3	17.6	14.7	NA
SEC	7.3	8.4	7.0	NA

Means in a column followed by the same letter are not significantly different, LSD,  $P = 0.05$ . (Means for samples collected 74 h after application were not significantly different.) Untreated control mortalities were less than 10% for each sample period. See Table 1 for formulation composition, NA, not applicable; CVC, critical value for comparison ( $P = 0.05$ ). SEC, standard error of comparison.

Table 6. Residual activity based on percentage mortality of *T. ni* when placed on cabbage leaves harvested after application of A/MNPV formulations at  $1.2 \times 10^{12}$  PIB/ha in 234 liters/ha to field-grown cabbage plants, Peoria, IL, 14 July 1997

Formulation	Hours after application			
	2	26	50	74
Lignin-flour	88.8	47.5b	18.4cd	1.7
Lignin-composite	94.2	80.1a	34.4ab	5.8
Lignin only	85.2	27.5c	11.8d	11.0
BBH-flour	85.0	52.5b	43.3a	10.9
BBH-composite	87.5	50.0b	29.0bc	13.8
BBH only	88.2	63.3ab	34.9ab	15.0
Unformulated	97.5	19.6c	13.4d	1.7
F (df = 6, 18)	1.1	13.1	7.0	1.6
CVC	NA	16.8	13.6	NA
SEC	NA	8.0	6.5	NA

Means in a column followed by the same letter are not significantly different, LSD,  $P = 0.05$ . (Means for samples collected 2 and 74 h after application were not significantly different.) Untreated control mortalities were less than 10% for each sample period. NA, not applicable. See Table 1 for formulation composition. CVC, critical value for comparison ( $P = 0.05$ ). SEC, standard error of comparison.

conditions. Within 2 d after application, most formulations were killing <20% of the larvae tested.

*A/MNPV.* No significant differences occurred in mortality among samples collected 2 h after application (Table 6). Loss of activity was observed within 26 h after application as only the lignin-composite formulation killed >75% *T. ni*. Lignin-composite killed significantly more larvae than the other formulations except for the Blankophor BBH only formulation. Within 3 d after application, very little activity remained in any formulation.

*July 1998 Application.* No rainfall occurred during this field test. Unformulated virus lost significant activity after just 6 h exposure in the field. No significant differences occurred among formulated A/MNPV until 26 h after application (Table 7), when the glycerin formulation lost significantly more activity than the

Table 7. Residual activity based on percentage mortality of *T. ni* when placed on cabbage leaves harvested after application of A/MNPV formulations at  $2.5 \times 10^{12}$  PIB/ha in 234 liters/ha to field-grown cabbage plants, Peoria, IL, 27 July 1998

Treatment	Hours after application				
	2	6	26	50	74
Lignin-flour	95.8	94.0a	91.6ab	75.8a	57.5ab
Lignin-flour-TiO <sub>2</sub>	92.5	95.7a	94.9ab	81.7a	49.2b
Lignin only	94.1	96.6a	96.7a	88.3a	65.0a
Lignin-TiO <sub>2</sub>	90.8	94.9a	86.6b	78.3a	56.4ab
Glycerin	98.3	96.6a	75.0c	54.5b	14.4c
Unformulated	86.7	39.4b	22.7d	15.1c	14.3c
F (df = 5, 15)	1.15	30.6	92.0	35.7	18.7
CVC	NA	12.5	8.9	13.8	15.8
SEC	NA	5.9	4.2	6.5	7.4

Means in a column followed by the same letter are not significantly different, LSD,  $P = 0.05$ . (Means for samples collected 2 h after application were not significantly different.) Untreated control mortalities were less than 10% for each sample period. NA, not applicable. See Table 1 for formulation composition. CVC, critical value for comparison ( $P = 0.05$ ). SEC, standard error of comparison.

**Table 8.** Residual activity based on percentage mortality of *T. ni* when placed on cabbage leaves harvested after application of A/MNPV formulations at  $2.5 \times 10^{12}$  PIB/ha in 234 liters/ha to field-grown cabbage plants, Peoria, IL, August 1998

Formulation	Hours after application				
	2	26	50	76	96
Lignin-flour	97.4a	80.0ab	52.5bc	48.3ab	27.0
Lignin-flour-TiO <sub>2</sub>	99.2a	75.6b	64.2abc	48.3ab	37.4
Lignin only	95.0a	88.3ab	65.1ab	62.5a	32.1
Lignin-TiO <sub>2</sub>	93.3a	80.0ab	71.7a	53.7a	19.4
Glycerin	100.0a	92.4a	46.1c	36.9bc	29.2
Unformulated	85.0b	36.6c	23.3d	21.8c	15.2
<i>F</i> (df = 5, 15)	5.2	18.4	7.9	7.8	1.5
CVC	7.3	14.1	18.9	15.3	NA
SEC	3.4	6.6	8.9	7.2	NA

Means in a column followed by the same letter are not significantly different, LSD,  $P = 0.05$ . (Means for samples collected 96 h after application were not significantly different.) Untreated control mortalities exceeded 10% for the 2 h sample (23.0% mortality), the 50 h sample (11.5% mortality) and the 76 h sample (20.8% mortality). NA, not applicable. See Table 1 for formulation composition. CVC, critical value for comparison ( $P = 0.05$ ). SEC, standard error of comparison.

others. This trend continued through the 74-h sample as well. The four lignin based formulations all still killed >50% of the test larvae following 3 d exposure in the field.

**August 1998 Application.** As in past tests, unformulated virus lost activity quickly (Table 8). The samples collected 26 h after application revealed that the glycerin formulation had higher activity than the lignin-flour-TiO<sub>2</sub> formulation but was not different from the rest of the lignin-based formulations. By 50 h after application, the lignin-only and lignin-TiO<sub>2</sub> formulations had significantly higher activity than the glycerin formulation. Again, the lignin-based formulation killed almost 50% of the test insects following 3 d field exposure. Within 4 d, no significant differences occurred among virus treatments. In this experiment, 18 mm rain was recorded between the 2- and 26-h sample collections and another 50 mm fell just after the 26-h sample was collected.

### Discussion

The formulation materials (Table 1) were selected based on previous experience with *B. thuringiensis* (Tamez-Guerra et al. 2000b) and nucleopolyhedroviruses (Tamez-Guerra et al. 2000a). Lignin (either potassium lignate, used in 1997, or sodium lignate, used in 1998) and flour 961 (a pregel corn flour) are at least partially soluble in cold water and will cross-link to form an insoluble material upon drying. Each of these materials has provided solar stability in past experiments. Optical brighteners similar to Blankophor BBH were reported (Shapiro and Robertson 1992, Li and Otvos 1999) to enhance activity of virus. The use of this material as a spray tank adjuvant has shown promise as an additive to virus preparations but has not been adopted due to cost and field handling issues. By using a small amount of Blankophor BBH encapsulated with the virus, we hoped to maintain the enhancement

factor and determine whether solar protection was afforded. Titanium dioxide was included in some of the formulations to determine its effect in providing solar stability to the viruses. TiO<sub>2</sub> is known to prevent sunburn by reflecting light energy rather than absorbing light energy as with optical brighteners or lignin, and had previously been tested by Bull et al. (1976) as a protectant for a *Heliothis* NPV.

The use of Blankophor BBH was stimulated by reports that optical brighteners extended residual activity as a result of protection from UV degradation (Nickle and Shapiro 1994, Dougherty et al. 1996, Webb et al. 1998) and enhancement of virus efficacy (Shapiro and Robertson 1992, Farrar et al. 1995). Our measurements for extended residual activity could result from either enhanced activity or protection from UV degradation. However, the relatively high cost of fluorescent brighteners (about \$20/kg) and their use as tank mix adjuvants (applied as a percentage of the spray volume) have hindered commercial acceptance. By using Blankophor BBH as a formulation ingredient in relatively low concentrations, we expected to see activity higher than in other formulations. Ac formulations were applied at 8 g BBH/ha for BBH-flour and BBH-composite formulations, and 1.4 kg for the BBH-only formulation (Table 5) and Af formulations were applied at 15, 32, and 450 g/ha for the BBH-flour, BBH-composite and BBH-only formulations, respectively (Table 6). Unfortunately, our initial activity tests and our field tests clearly did not reveal an improvement in activity or residual activity. Other laboratory tests confirmed this observation (Tamez-Guerra et al. 2000a). Subsequently, Blankophor BBH was dropped from further testing in 1998. This result may not be representative of all brighteners as suggested by the variable results among brighteners reported previously (Shapiro 1992, Li and Otvos 1999). M. Shapiro (personal communication) demonstrated that calcium ions complex and interfere with Blankophor's activity. Our dried formulations use calcium ion for crosslinking and would neutralize Blankophor. Thus, the lack of enhancement by Blankophor in our tests supports observations made by Shapiro.

Titanium dioxide is known to reflect light energy and is commonly used in many products including foods, paints and sunscreens. Bull et al. (1976) included TiO<sub>2</sub> as an ingredient in formulations with favorable results. In addition, to providing solar protection, TiO<sub>2</sub> is used for photocatalytic degradation of contaminants in liquid slurries (Hustert et al. 1991, Mak and Hung 1992, Pugh et al. 1995) because it produces reactive OH radicals. These reactive radicals may be detrimental for virus, and Shapiro (1995) suggested that radical scavengers may be important in protection of insect pathogens during solar irradiation. Thus, the effect of TiO<sub>2</sub> as a component of dried formulations is unclear, as these formulations containing TiO<sub>2</sub> did not have a great benefit or detriment expressed as residual activity.

There is a need for improved residual activity of entomopathogenic virus formulations, and scientists

are striving for 7- to 10-d residual activity. In our tests, unformulated virus began to lose activity within hours after application and generally averaged 32% original activity (time 2 h) remaining by 26 h after application (averaged for 1997 and 1998 tests with AfMNPV conducted in Illinois). A formulation consisting of glycerin lost  $\approx 50\%$  activity within 2 d after application and represents longer residual activity compared with unformulated virus (1998 tests conducted in Illinois). Glycerin is currently used in formulations of virus marketed by Thermo Trilogy for the control of *Spodoptera exigua* (Hübner) and *Anticarsia gemmatilis* (Hübner) and represents the standard commercial formulation. Longer residual activity of AfMNPV and AcMNPV were observed, however, when various additives were spray dried together with the virus. In Mississippi cotton, lignin-based formulations maintained  $>40\%$  mortality longer after application than the other formulations tested. In Illinois cabbage tests, residual activity in 1998 was longer with lignin-based formulations of AfMNPV, but was less obvious compared with results from 1997. In both 1998 tests in Illinois,  $>50\%$  of the test insects died after feeding on cabbage leaves that had been treated with lignin-based formulations of virus applied 3 d earlier.

The use of a spray dryer to prepare formulations of entomopathogens has long been used to recover *B. thuringiensis* from fermentation vessels and is used by many commercial producers. Tamez-Guerra et al. (2000b) demonstrated that formulation ingredients could be spray dried with *B. thuringiensis* to extend residual field activity. With viruses, spray dried lignin-based formulations successfully encapsulate the PIBs and retain insecticidal activity longer than unformulated virus after application. Care must be taken with spray dry conditions and formulation ingredients to avoid losing significant insecticidal activity. The results presented here and elsewhere (Tamez-Guerra et al. 2000a) are the culmination of many trials with different ingredients and spray dry conditions demonstrating the versatility of this formulation process. In addition to residual activity issues, parameters such as shelf life, mixing, acceptability and digestibility by insects, application problems, and loading rates still need to be considered. Improvements are necessary and work is continuing on these factors.

### Acknowledgments

We thank Erica Bailey, Holly Goebel, and Monica Wetzel for excellent technical assistance. We also thank Thermo Trilogy and DuPont for supplying virus and Martin Shapiro for supplying Blankophor BBH.

### References Cited

- Anagrapha falcifera nucleopolyhedrovirus. J. Invertebr. Pathol. 76: 120–126.
- Bull, D. L., R. L. Ridgway, V. S. House, and N. W. Pryor. 1976. Improved formulations of the *Heliothis* nuclear polyhedrosis virus. J. Econ. Entomol. 69: 731–736.
- Dougherty, E. M., K. P. Guthrie, and M. Shapiro. 1996. Optical brighteners provide Baculovirus activity enhancement and UV radiation protection. Biol. Control 7: 71–74.
- Farrar, R. R., Jr., R. L. Ridgway, S. P. Cook, K. W. Thorpe, and R. E. Webb. 1995. Nuclear polyhedrosis virus of the gypsy moth (Lepidoptera: Lymantriidae): potency and effects of selective adjuvants on insect feeding behavior. J. Entomol. Sci. 30: 417–428.
- Finney, D. J. 1971. Probit analysis, 3rd ed. Cambridge University Press, Cambridge, MA.
- Hughes, P. R., and H. A. Wood. 1981. A synchronous peroral technique for the bioassay of insect viruses. J. Invertebr. Pathol. 37: 154–159.
- Hustert, K., P. N. Moza, and B. Pouyet. 1991. Photocatalytic degradation of s-triazine herbicides. Toxicol. Environ. Chem. 31/32: 97–102.
- Ignoffo, C. M., B. S. Shasha, and M. Shapiro. 1991. Sunlight ultraviolet protection of the *Heliothis* nuclear polyhedrosis virus through starch-encapsulation technology. J. Invertebr. Pathol. 57: 134–136.
- Ignoffo, C. M., C. Garcia, and S. G. Saathoff. 1997. Sunlight stability and rain-fastness of formulations of baculovirus *Heliothis*. Environ. Entomol. 26: 1470–1474.
- Jakes, R. P. 1977. Stability of entomopathogenic viruses, pp. 99–116. In C. M. Ignoffo and D. L. Hostetter [eds.], Environmental stability of microbial insecticides. Misc. Publ. Entomol. Soc. Am. 10.
- Li, S. Y., and I. S. Otvos. 1999. Comparison of the activity enhancement of baculovirus by optical brighteners against laboratory and field strains of *Choristoneura occidentalis* (Lepidoptera: Tortricidae). J. Econ. Entomol. 92: 534–538.
- Mak, M.K.S., and S. T. Hung. 1992. Degradation of neat and commercial samples of organophosphate pesticides in illuminated TiO<sub>2</sub> suspensions. Toxicol. Environ. Chem. 36: 155–168.
- McGuire, M. R., D. A. Streett, and B. S. Shasha. 1991. Evaluation of starch encapsulation for formulation of grasshopper (Orthoptera: Acrididae) entomopoxviruses. J. Econ. Entomol. 84: 1652–1656.
- McGuire, M. R., L. J. Galán-Wong, and P. Tamez-Guerra. 1997. Bacteria: bioassay of *Bacillus thuringiensis* against Lepidopteran larvae, pp. 91–99. In L. Lacey [ed.], Manual of techniques in insect pathology. Academic Press, San Diego, CA.
- Nickle, W. R., and M. Shapiro. 1994. Effects of eight brighteners as solar radiation protectants for *Steinernema carpocapsae*, all strain. J. Nematol. 26: 782–784.
- Pugh, K. C., D. J. Kiserow, J. M. Sullivan, and J. H. Grinstead, Jr. 1995. Photocatalytic destruction of atrazine using TiO<sub>2</sub> mesh, pp. 174–194. In Emerging technologies in hazardous waste management V. ACS Symposium Series 607. American Chemical Society, Washington, DC.
- Shapiro, M. 1992. Use of optical brighteners as radiation protectants for gypsy moth (Lepidoptera: Lymantriidae) nuclear polyhedrosis virus. J. Econ. Entomol. 85: 1682–1686.
- Shapiro, M., and J. L. Robertson. 1992. Enhancement of gypsy moth, (Lepidoptera: Lymantriidae) baculovirus activity by optical brighteners. J. Econ. Entomol. 85: 1120–1124.
- Shapiro, M. 1995. Radiation protection and activity enhancement of viruses. ACS Symposium Series, Biora-
- Analytical Software. 1997. Statistix for Windows, user's manual. Analytical Software, Tallahassee, FL.
- Behle, R. W., M. R. McGuire, and P. Tamez-Guerra. 2000. Effect of light energy on alkali released virions from

- tional pest control agents formulation and delivery, pp. 153–164. National Meeting of the American Chemical Society, 13–17 March 1994, San Diego, CA. ACS, Washington, DC.
- Tamez-Guerra, P., M. R. McGuire, H. Medrano-Roldán, L. J. Galán-Wong, B. S. Shasha, and F. E. Vega. 1996. Sprayable granule formulations for *Bacillus thuringiensis*. J. Econ. Entomol. 89: 1424–1430.
- Tamez-Guerra, P., C. García-Gutiérrez, H. Medrano-Roldán, L. J. Galán-Wong, and C. F. Sandoval-Coronado. 1999. Spray dried microencapsulated *Bacillus thuringiensis* formulations for the control of *Epilachna varivestis* Mulsant. Southwest. Entomol. 24: 37–48.
- Tamez-Guerra, P., M. R. McGuire, R. W. Behle, J. J. Hamm, H. R. Sumner, and B. S. Shasha. 2000a. Sunlight persistence and rainfastness of spray-dried formulations of the *Anagrapha falcifera* baculovirus. J. Econ. Entomol. 93: 210–218.
- Tamez-Guerra, P., M. R. McGuire, R. W. Behle, B. S. Shasha, and L. J. Galán-Wong. 2000b. Assessment of microencapsulated formulations for improved residual activity of *Bacillus thuringiensis*. J. Econ. Entomol. 93: 219–225.
- Webb, R. E., N. H. Dill, J. M. McLaughlin, L. S. Kershaw, J. D. Podgwaite, S. P. Cook, K. W. Thorpe, R. R. Farrar, Jr., R. L. Ridgway, and R. W. Fuester. 1996. Blankophor BBH as an enhancer of nuclear polyhedrosis virus in arborist treatments against the gypsy moth (Lepidoptera: Lymantriidae). J. Econ. Entomol. 89: 957–962.
- Webb, R. E., R. Peiffer, R. W. Fuester, K. W. Thorpe, L. Calabrese, and J. M. McLaughlin. 1998. An evaluation of residual activity of traditional, safe, and biological insecticides against the gypsy moth. J. Arboric. 24: 286–293.

Received for publication 31 May 2000; accepted 14 February 2001.

---